



SPECIFICATION



TITLE OF INVENTION

Method of analysis of carboxylic acid by mass spectrometry

INVENTORS :

Hoa D. Nguyen and Trinh D. Nguyen

DATE SUBMITTED :

January 22, 2008.

CROSS-REFERENCE TO RELATED APPLICATIONS

Not applicable

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR
DEVELOPMENT

Not applicable

BACKGROUND OF THE INVENTION

This invention pertains to methods of quantitative analysis of carboxylic acids in a sample by isotope dilution mass spectrometry. The stable isotope labeled esters are used as internal standards. The sample may be a biological fluid, such as serum, urine etc., or an aqueous sample such as an environmental or an agricultural sample.

While various methods of analysis such as immunoassays and chromatographic analysis - LC (liquid chromatography), GC (gas chromatography), and TLC (thin layer chromatography) - have been reported for identification and determination of levels of carboxylic acids in analytical samples, the absolute and unequivocal identification and quantitative analysis of those compounds are combinations of chromatographic analysis and MS (mass spectrometry) such as GC-MS and LC-MS. The accuracy and precision of these methods are usually the highest when stable isotope analogs of the analytes are used as internal standards.

The mass spectrometry method of analysis using stable isotope labeled internal standards is commonly called isotope dilution mass spectrometry analysis. This method takes advantage of the similar chemical and physical behaviors of analytes, the chemical compounds being analyzed, and their respective isotope labeled internal standards towards all phases of sample preparation and also towards instrument responses. It uses the mass differentiation between analytes and their respective internal standard in mass spectrometry for quantification. The requirement for this method of analysis is the availability of stable isotope labeled internal standards.

The commonly used stable isotope labeled internal standard of an analyte is a chemical compound that has the same chemical structure as that of the analyte except that one or more

substituent atoms are stable isotopes. Four commonly used stable isotopes are deuterium, carbon-13, nitrogen-15, and oxygen-18. For every hydrogen atom that is replaced by a deuterium atom, the molecular weight of resulting chemical compound is increased by one mass unit. This is also true for replacing a carbon atom with a carbon-13 atom, or by replacing a nitrogen atom with a nitrogen-15 atom. In the case of replacing an oxygen atom with an oxygen-18 atom, the molecular increase is two mass units. Although the acceptable stable isotope labeled internal standard for isotope dilution mass spectrometry method is the one that is not contaminated with any of the unlabeled material, the ideal one should be the one with the highest isotopic purity and contains as many stable isotope atoms as possible. The ideal one, however, must not contain any labeled isotope that can be exchanged for the unlabeled isotope under particular sample preparation conditions.

These criteria of an ideal stable isotope labeled internal standard present a challenge for organic synthesis chemists who help the analytical chemists in the analysis. Most often the synthesis of stable isotope internal standards is not simply an isotope exchange reaction. Easily exchangeable atoms are usually avoided due to possible re-exchange during sample preparation steps. Organic chemists often have to carry out multi-step synthesis to make stable isotope labeled internal standards. Even though many stable isotope labeled reagents are commercially available, the choice of appropriate labeled reagent for chemical synthesis of stable isotope labeled internal standards is still very limited. The limited isotope labeled reagents and the multi-step synthesis contribute to the high cost of synthesis of stable isotope internal standards. Even if the analytical chemist who carries out the analysis can afford the cost of the synthesis, there is also a time factor that he or she has to consider before ordering the synthesis. Situations where

organic chemists spent weeks and months on a synthesis project and came up with nothing at the end were common. This invention offers a solution for this problem.

[The objective is a short and reliable method of preparing a stable isotope labeled internal standard that is suitable for the MS analysis of an analyte in question, but not the synthesis of the stable isotope labeled analyte. Within the context of the isotope dilution mass spectrometry method, both analyte and its internal standard have to have identical chemical structures, with the exception of the isotope atoms which provide the mass differentiation upon mass spectrometric analysis.] Analytical chemists who uses GC-MS for their analysis often “derivatize” the analyte and its stable isotope labeled [analyte (used as) internal standard (s)] into chemical compounds that can easily pass through the GC column or else provide better instrumental responses. The MS analysis becomes the analysis of the “derivatized[” analyte and the “derivatized”] chemical compounds” of both the analyte and the labeled internal standard, but still provides comparably accurate results of concentrations of the analyte itself. Examples of [these analyses] “derivatization method” are found in cited references. An example of using a derivatization method is found in the GC-MS analysis of homocysteine by Jens Pietzsch et al. A patient sample containing carboxylic acid homocysteine was treated with a “derivatizing reagent” ethyl alcohol in the presence of an activating reagent ethyl chloroformate and a base pyridine to convert homocysteine to a “derivatized chemical compound” called homocysteine ethyl ester. Homocysteine-d4 was used as internal standard for the MS analysis. It was added to the sample before sample processing and was also derivatized to a “derivatized chemical compound” called homocysteine ethyl ester-d4. After derivatization, both “derivatized chemical compounds”, homocysteine ethyl ester and homocysteine ethyl ester-d4, are isolated by an aqueous extraction and are analyzed by GC-MS. The analysis provided ion signals of the esters which were used for

the determination of the concentration of the carboxylic acid homocysteine. The requirement for MS analysis in this case, or in isotope dilution MS analysis in general, is that both chemical compounds introduced to MS instrument have to have identical chemical structures with the exception of the labeled isotope atoms, whether they are derivatized or not. This requirement is the fundamental basis of isotope dilution MS analysis. The analysis of homocysteine by Jens Pietzsch et al. was possible with an availability of homocysteine-d4. The methods of the present invention are directed to the synthesis of “derivatized chemical compounds” that are useful in the isotope dilution MS analysis of carboxylic acid where there is unavailable supply of labeled carboxylic acid such as homocysteine-d4. This usefulness of the “derivatized chemical compounds” follows the fundamental basis of isotope dilution MS analysis. [Using similar reasoning, one can synthesize a stable isotope derivative of the analyte by reacting it with a stable isotope labeled reagent. The resulting isotope labeled chemical compound can be used as internal standard in the analysis of the analyte, providing that the analyte in the analyzed sample will be converted to a chemical compound of identical structure as that of the internal standard using a non-labeled reagent. There are 3 requirements for the usefulness of this method :

1. The analyte in the sample must be *quantitatively* converted to the compound of identical structure (except the labeled atoms) as that of the added isotope labeled internal standard using a non-labeled reagent.
2. Absolutely no conversion of the isotope labeled internal standard to the non-labeled compound because the conversion of the analyte happens in the sample in the presence of the added isotope labeled internal standard.
3. The conversion of the analyte into the compound of identical structure as that of the added

isotope labeled internal standard has to be accomplished before any isolation method i.e. extraction, is performed.

The first two requirements relate to the chemistry of the analyte in question. The efficiency of a chosen chemical reaction depends on the type of reaction which, in turn, depends on the type of functional groups of the analyte. This invented method relates to the analysis of carboxylic acids whose chemistry focus on the reactivity of the carboxyl functional groups of the analyte.

Quantitative reactions of carboxylic acids in aqueous samples are :

1. ——— Conversion to an ester using a chloroformate and an alcohol.
2. ——— Conversion to an ester using an alkyl halide under alkaline conditions.

There are other reactions of carboxylic acids that are very efficient, but the above conversion reactions are very efficient in aqueous environment and can be performed at room temperature and in a relatively short reaction time. These are necessary and practical features for routine analysis of carboxylic acids in aqueous samples.]

BRIEF SUMMARY OF THE INVENTION

The objective is a short and reliable method of preparing stable isotope labeled internal standard that is suitable for the MS analysis of an aqueous sample containing unknown concentration of carboxylic acid but not the synthesis of the stable isotope labeled carboxylic acid. The current invention provides for a method of identification and quantification of carboxylic acid in a sample as carboxylic acid ester by isotope dilution mass spectrometry . The stable isotope labeled internal standard of said carboxylic acid is synthesized beforehand by reacting [a] an authentic sample [containing] of said [analyzed] carboxylic acid with a labeled

reagent having at least one enriched stable isotope atom called "labeled derivatizing reagent". Following this step, said stable isotope labeled internal standard is then added to said sample containing said [analyzed] carboxylic acid. Said [analyzed carboxylic acid] sample is then ~~[converted to]~~ treated with a non labeled [analog] version of said labeled derivatizing reagent to convert only the carboxylic acid to carboxylic acid ester [said labeled internal standard with identical chemical structure as said labeled internal standard except for the stable isotope atoms using a non-labeled reagent]. Both said [converted] carboxylic acid ester and its corresponding stable isotope labeled carboxylic acid ester internal standard are then extracted and analyzed by mass spectrometry for the determination of the concentration of said carboxylic acid in said sample. ~~[Said stable isotope labeled internal standard provided in the current invention are labeled carboxylic acid ester analogs of said analyzed carboxylic acid. There are 2 methods to quantitatively convert a carboxylic acid to a carboxylic acid esters under aqueous conditions, called 100% conversion. One method requires a chloroformate to activate the acid to form an intermediate activated ester which reacts with an added alcohol to form the desired carboxylic acid ester. The other method requires a strong alkaline condition for the carboxylic acid to react with an added alkyl halide to form the desired carboxylic acid ester.]~~

~~———— In comparison with the traditional method of isotope dilution mass spectrometric analysis of more than one carboxylic acids, the invented method offers the following advantages:~~

- ~~1. ——— The efficiency and simplicity of the above reactions makes possible the short, reliable, and quick synthesis of individual stable isotope labeled internal standards, whereas in the traditional method of analysis, stable isotope labeled internal standard of each carboxylic acid has to be independently synthesized.~~

2. ~~It is possible to quickly and efficiently synthesize a library of stable isotope internal standards for the analysis of an entire library of carboxylic acids using these reactions and only one commercially available stable isotope labeled reagent.~~

3. ~~Because the synthesis of stable isotope labeled internal standard in this invented method is usually a one step synthesis, the entire process of synthesis and sample preparation can be performed in an automated fashion. The internal standard is prepared in one step, excess isotope reagent is then destroyed or removed, and the prepared internal standard can be added directly to the samples without purification. The non-labeled reagent is added and the sample is ready for extraction shortly thereafter.~~

~~These attractive features make the method suitable for high throughput analysis of carboxylic acids by isotope dilution mass spectrometry.]~~

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates a mass spectrum of the synthesized ketoprofen ethyl ester-d5 in molecular ion mode.

Figure 2 illustrates a mass spectrum of the synthesized ketoprofen ethyl ester-d5 in multiple reaction monitoring mode.

Figure 3 illustrates a mass spectrum of the derivatized ketoprofen ethyl ester in molecular ion mode.

Figure 4 illustrates a mass spectrum of the derivatized ketoprofen ethyl ester in multiple reaction monitoring mode.

Figure 5 illustrates a mass spectrum of the extract of a sample containing the derivatized ketoprofen ethyl ester and the synthesized labeled internal standard ketoprofen ethyl ester-d5 in multiple reaction monitoring mode.

DETAILED DESCRIPTION OF THE INVENTION

The current invention provides [~~for a~~] methods of one step synthesis of stable isotope labeled internal standards and additional chemical reaction for the purpose of identification and quantification of carboxylic acid(s) in [a] an aqueous sample by mass spectrometry analysis. Said

carboxylic acid(s) has the following formulas $R_1\text{COOH}$ wherein R_1 is alkyl, aryl, heteroatom containing, cyclic or non-cyclic groups. The current method comprises, as an integral part of said analysis of said carboxylic acid(s), the following steps:

1. Synthesizing labeled carboxylic acid ester internal standard(s) by reacting an authentic sample of said carboxylic acid(s) with a stable isotope labeled reagent having at least one enriched stable isotope atom called "labeled derivatizing reagent" to form [said] labeled carboxylic acid ester[~~-internal standard~~](s) of the general formulas $R_1\text{COOR}_2$, wherein R_2 is a stable isotope labeled alkyl group coming from the labeled derivatizing reagent [~~Said R_2 stable isotope labeled alkyl group is selected from the group consisting of~~] such as CD_3 or CD_2CD_3 or $\text{CD}_2\text{C}_6\text{D}_5$. [~~Depending on the method mentioned above to~~] To convert a carboxylic acid to a carboxylic acid ester, said stable isotope labeled derivatizing reagent is either [a chloroformate and] a labeled alcohol [selected from the group consisting of labeled methanol and labeled ethanol,] or [a base and] a labeled alkyl halide [selected from the group consisting of labeled methyl iodide, labeled ethyl iodide, and labeled benzyl chloride]. Both labeled alcohol and labeled alkyl halide react preferably in the presence of an activating reagent such as ethyl chloroformate or methyl chloroformate or butyl chloroformate and a base such as pyridine or sodium hydroxide or sodium carbonate. The labeled alcohol, represented as $R_2\text{OH}$ wherein R_2 is a labeled alkyl group, includes labeled alcohols such as methanol-d3 (CD_3OH) or ethyl-d5 alcohol ($\text{CD}_3\text{CD}_2\text{OH}$) or benzyl alcohol-d7 ($\text{C}_6\text{D}_5\text{CD}_2\text{OH}$). The labeled alkyl halide, represented as $R_2\text{X}$ wherein R_2 is a labeled alkyl group, includes labeled alkyl halides such as methyl iodide-d3 (CD_3I) or ethyl-d5 iodide ($\text{CD}_3\text{CD}_2\text{I}$) or benzyl chloride-d7 ($\text{C}_6\text{D}_5\text{CD}_2\text{Cl}$).

2. A known amount of said stable isotope labeled ester internal standard(s) was then added to said sample containing unknown concentration of said carboxylic acid(s) to be analyzed.
3. Said resulting sample was then [~~contacted~~] treated with [~~either~~] a [~~chloroformate such as isobutylchloroformate and a~~] non-labeled [~~alcohol selected from a group consisting of methanol and ethanol, or a strong base such as sodium hydroxide and an alkyl halide selected from a group consisting of methyl iodide, ethyl iodide, and benzyl chloride,~~] version of said labeled derivatizing reagent to quantitatively convert said carboxylic acid(s) in said sample into said carboxylic acid ester(s) of identical structure as that of said carboxylic acid ester internal standard(s) except for the stable isotope atoms. Said carboxylic acid ester(s) has the the general formulas R_1COOR_2 , wherein R_2 is a non-labeled alkyl group coming from the non-labeled derivatizing reagent. The additional reaction of said carboxylic acid(s) in said sample with said non-labeled derivatizing reagent follows the same reaction conditions as those of the reaction of the labeled derivatizing reagent which include the use of same activating reagent, same base, and same non-labeled derivatizing reagent alcohol or alkyl halide. Said alcohol includes such alcohols as methanol, ethanol, and benzyl alcohol while said alkyl halide includes such alkyl halides as methyl iodide, ethyl iodide, and benzylchloride. Even though the reaction of alcohol or alkyl halide with carboxylic acids in the presence of an activating reagent and a base is generally an irreversible reaction, it is important to work out reaction conditions so that the labeled carboxylic acid ester internal standard(s) in said sample is unreactive or does not convert itself to the corresponding carboxylic acid ester. It is also important to work out reaction conditions (i.e. using excess derivatizing reagent) so that

- the reaction of said derivatizing reagent with said carboxylic acid(s) be “quantitative” meaning all carboxylic acid(s) are completely converted to carboxylic acid ester(s)
4. Appropriate aqueous extraction methods were then used to isolate said ester(s) and ~~[their]~~ said corresponding ester internal standard(s) from said sample.
 5. Concentration of said ester(s) were determined and quantified by mass spectrometry analysis of the extract ~~[and based on the ratio of said converted carboxylic acid ester(s) and said corresponding carboxylic acid ester internal standard]~~.
 6. Samples containing known concentration of said carboxylic acid(s) were treated with the same amount of labeled carboxylic acid ester internal standard(s) and are processed the same way as said sample of unknown concentration of carboxylic acid(s) for the purpose of construction of a calibration curve.
 7. Concentration of carboxylic acid(s) in said sample can be reliably deduced from said calibration curve.

Example : Analysis of Ketoprofen in human plasma.

Ketoprofen is an anti-inflammatory drug having a carboxyl function. Its chemical name is 2-(3-benzoyl-phenyl)propionic acid. MS analysis of ketoprofen in patient plasma samples may be achieved by using labeled ketoprofen as internal standard. This invention provides methods of synthesis of labeled ketoprofen ester that can be used as labeled internal standards in the MS analysis of ketoprofen in patient samples in case where labeled ketoprofen is not commercially available or in case where its synthesis requires multi step reactions. The synthesis of labeled ketoprofen ethyl ester can be accomplished in one step by reaction of an authentic sample of ketoprofen and a labeled ethyl alcohol such as ethyl-d5 alcohol. The use of labeled ketoprofen ethyl ester (ketoprofen ethyl ester-d5) as labeled internal standard requires

an additional reaction to be performed on samples. This additional reaction is the reaction of ketoprofen in samples with ethyl alcohol, a non-labeled version of the derivatizing reagent used for the synthesis of labeled ketoprofen ethyl ester. When the reaction of the ethyl alcohol and ketoprofen is performed under aqueous conditions such as in patient samples, the use of an activating reagent ethyl chloroformate and a base pyridine in addition to an excess of ethyl alcohol is necessary to obtain quantitative reaction. However, when the reaction is performed under non-aqueous conditions for the purpose of preparing large quantity of ketoprofen ethyl ester, the use of an activating reagent dicyclohexyl carbodiimide is preferred to ethyl chloroformate and pyridine. The required chemicals for the analysis include an authentic sample of ketoprofen, ethyl chloroformate, ethanol, and ethyl-d5 alcohol (or ethanol-d5). Concentration of ketoprofen in plasma samples designated as samples J,K, and L are determined by MS analysis in parallel with the MS analysis of plasma samples containing known concentration of ketoprofen designated as A to G (see below). For the sake of verifying the performance of the invention method, plasma samples J,K, and L are made at specific concentration and these concentrations are to be compared with results obtained for these plasma samples from the calibration curve.

Method : Before processing of plasma samples, an authentic sample of ketoprofen is derivatized to ketoprofen ester-d5 by reaction with ethyl chloroformate and ethanol-d5. Then known equal volumes of a solution of ketoprofen ester-d5 are added to plasma samples A to G, and also J,K,L. These samples are then derivatized by reaction with ethyl chloroformate and ethanol. Ketoprofen in these plasma samples are derivatized to ketoprofen ester while the added internal standard ketoprofen ester-d5 remained unreacted. The plasma samples are then subjected to an aqueous extraction using ethyl acetate as the organic extraction solvent. The extracts are analyzed by MS in multiple reaction monitoring (MRM) mode which makes use of a combination of molecular ion and product ion (or daughter ion). Molecular ion is the ion generated from the ionization of the molecular mass of a chemical compound. Product ion or daughter ion is the ion generated from the additional ionization of the molecular ion. The MS analysis

in MRM mode provides the most selective analysis, especially when there are presence of chemical compounds having the same molecular mass.

Step 1 : Preparation of Ketoprofen ethyl ester-d5.

A solution of 25 mg of ketoprofen in 0.5ml tetrahydrofuran was treated with 2 equivalents of ethanol-d5 and one equivalent dicyclohexyl carbodiimide. The resulting solution was stirred for 20 hours then was quenched with water. The aqueous phase was extracted with ethyl acetate and the combined organic phases were dried with magnesium sulfate. The filtered solution was concentrated and the residue was purified by column chromatography using silica gel as absorbant and hexane ethyl acetate mixture as eluant. The fractions containing clean ketoprofen ethyl ester-d5 were combined and concentrated to give 8mg product as a white solid. MS analysis gave MH+ 288.

Step 2 : Preparation of working standard solutions and internal standard solution.

Working standard solutions of ketoprofen were prepared by weighing ketoprofen and diluting the stock solution to appropriate concentration as follows :

Solution A	0.1 ug/ml in ethyl acetate
B	0.2 ug/ml
C	0.5 ug/ml
D	2.0 ug/ml
E	5.0 ug/ml
F	15.0 ug/ml
G	20.0 ug/ml

Working quality control standard solutions of ketoprofen were prepared by independently weighing ketoprofen and diluting the stock solution to appropriate concentration as follows :

QC Solution J	0.3 ug/ml in ethyl acetate
K	6.0 ug/ml
L	14.0 ug/ml

Working internal standard solution of ketoprofen were prepared by weighing ketoprofen ethyl ester-d5 and diluting the stock solution to a working concentration of 10 ug/ml in ethyl acetate.

Step 3 : Preparation of calibration samples and quality control samples in human plasma.

Ketoprofen-free human plasma aliquots of 0.1ml were treated with 100ul of solution A to G to make calibration samples A to G.

Ketoprofen-free human plasma aliquots of 0.1ml were treated with 100ul of solution J to L to make quality control samples J to L.

Both calibration samples and quality control samples were then treated with 100ul of the internal standard working solution.

A human plasma aliquot of 0.1ml was treated with 100ul of the internal standard solution to make the "zero" sample.

Another human plasma aliquot of 0.1ml was not treated with 100ul of the internal standard solution to make the "blank" sample.

Step 4 : Ester formation and extraction.

To all prepared samples were added 100ul of a solution of water:ethanol:pyridine (60:32:8) followed by 10ul of ethyl chloroformate. The samples were mixed and left standing at room temperature for 15 minutes. Aqueous 1N hydrochloric acid, 0.5ml, was added to each sample and they were extracted with 0.5ml ethyl acetate. Each extract was separated and concentrated. The residue of each extract was reconstituted with 100ul of acetonitrile.

Step 5 : Analysis of reconstituted extracts by LC/MS/MS.

A total of 12 reconstituted extracts were loaded on a Perkin Elmer autosampler that was connected to a Perkin Elmer LC pump and a PE Sciex API 365 MS. Each extract was run through an Inersil column of 5um at a rate of 0.5ml/min of acetonitrile/water 50/50 mixture. The eluate was directly fed to the MS ion source. MS data were collected for 1.5min per injection.

MS analysis was performed in MRM mode. m/z 283.0 > m/z 209.0 was monitored for ketoprofen ethyl ester while m/z 288.0 > m/z 209.0 was monitored for ketoprofen ethyl ester-d5. Collected data were plotted against concentration using McQuan 1.5 software.

Results are tabulated as follows:

Ketoprofen

Internal Standard: is

Weighted ($1/x^2$)

Intercept = 0.030

Slope = 0.040

Correlation Coeff. = 0.996

Use Area

<i>Filename</i>	<i>Accuracy</i>	<i>Conc.</i>	<i>Calc. Conc.</i>	<i>Int. Ratio</i>
Keto A Standard	93.212	0.100	0.093	0.034
Keto B Standard	108.585	0.200	0.217	0.039
Keto C Standard	114.109	0.500	0.571	0.053
Keto D Standard	95.505	2.000	1.910	0.107
Keto E Standard	97.619	5.000	4.881	0.225
Keto F Standard	94.386	15.000	14.158	0.596
Keto G Standard	96.583	20.000	19.317	0.802
Keto J QC	104.298	0.300	0.313	0.043
Keto K QC	98.680	6.000	5.921	0.267
Keto L QC	100.604	14.000	14.085	0.593

Results : A calibration curve for ketoprofen in the range of 0.100 ng/ml to 20.000 ng/ml in human plasma was constructed from MS data obtained for calibration samples A to G. From this calibration curve, concentration of ketoprofen in control plasma samples J(0.300ng/ml), K(6.000ng/ml), and K(14.000ng/ml) are deduced as 0.313 ng/ml, 5.921 ng/ml, and 14.085 ng/ml respectively. It is noted that the MS data obtained for all samples are of ion signals of ketoprofen ester. However, ketoprofen in all samples are derivatized the same way. Therefore, concentration of ketoprofen in all samples can be correctly determined from the calibration curve of ketoprofen ester.

While the invention is particularly described with the illustrated example, it will be understood by those skilled in the art that the foregoing and other changes in details may be made therein without departing from the scope of the invention.

REFERENCES

US patent documents

5,559,038 September 24, 1996 J. Fred Kolhouse

6,358,996 March 19, 2002 Michael S. Alexander

Other references

Petr Husek, "Chloroformates in gas chromatography as general purpose derivatizing agents",
Journal of Chromatography B, 1998, page 57-91, vol. 717.

Jens Pietzsch et al, "Rapid determination of total homocysteine in human plasma by using
N(O,S)-Ethoxycarbonyl ethyl ester derivatives and gas chromatography-mass spectrometry",
Clinical Chemistry, 1997, page 2001-2004, vol. 43(10).

Petr Husek and Petr Simek, "Advances in amino acid analysis", LCGC Sep 2001, page 986-999,
vol.19.

Ping Cao and Mehdi Moini, "Quantitative analysis of fluorinated ethylchloroformate derivatives
of protein amino acids and hydrolysis products of small peptides using chemical ionization gas

chromatography-mass spectrometry”, Journal of Chromatography A, 1997, page 111-117, vol. 759.

William A. Joern, “Unexpected volatility of barbiturate derivatives : an extractive alkylation procedure for barbiturates and benzoylecgonine”, Journal of Analytical Toxicology, Nov 1994 page 423, vol. 18.